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# Evaluation of the Francome MkII Exhaust Nozzle Sprayer to Apply Oil-Based Formulations of *Metarhizium flavoviride* for Locust Control

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**Abstract:** Conidia of the fungus *Metarhizium flavoviride* were formulated in a paraffinic oil, 'Shellsol' T, and sprayed using the Francome MkII exhaust nozzle sprayer. Germination of the conidia collected from the spray was reduced by 30% as compared to unsprayed conidia. However, in bioassays, there was no detectable difference in virulence with conidia collected from the spray samples and unsprayed formulation. This indicated that, despite the recorded reduction in the concentration of active conidia, the efficacy of the formulation remained unchanged after passing through the exhaust nozzle sprayer.

The droplet size spectra produced by the sprayer were investigated using the Malvern series 2600cc particle size analyser. The optimum droplets for locust control produced by this sprayer were generated by the number 1 nozzle (internal diameter 2·5 mm) with the number 1 restrictor ring (internal diameter 12.5 mm) sprayed at a pressure of 0·2 bar. The droplets thus produced had a volume median diameter of 58 $\mu$m when the nozzle protruded between 1 and 2 mm above the level of the restrictor ring. Of the droplets in the spray plume created by these conditions, 33% were between 50 and 100 $\mu$m, a range recommended as an achievable optimum for the ultra-low-volume application of *Metarhizium flavoviride*. The role of the exhaust nozzle sprayer as a tool for the application of *M. flavoviride* for locust control is discussed with reference to other vehicle-mounted ultra-low-volume sprayers.

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Key words: *Metarhizium flavoviride*, exhaust nozzle sprayer, droplet spectra, ultra-low-volume, locust control

## 1 INTRODUCTION

Concern over the harmful effects of the pesticides used for the control of locusts, in particular the desert locust *Schistocerca gregaria* (Forskål),[1] brought about several research programmes to develop biocontrol agents against locusts and grasshoppers.[2] The LUBILOSA† project is directed towards the development of fungal pathogens to be applied as biopesticides for locust and grasshopper control and in particular is focusing upon

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the use of *Metarhizium flavoviride* Gams & Rozsypal for this purpose.[3,4] The programme has taken a 'step-by-step' approach to application issues, with a diversification of techniques and an increase in the scale of trials following initial success with hand-held equipment.[5]

The Exhaust Nozzle Sprayer (ENS) was first used in 1953 to spray settled swarms of locusts in bush country in Kenya.[6] Atomisation of the spray is brought about by forcing the exhaust gases of a petrol or diesel engine through the vertical exhaust stack of the sprayer to a restricted orifice surrounding a jet of insecticide formulation. Back pressure caused by restriction of the exhaust gases at the orifice of the exhaust stack is directed to the tank containing the formulation, putting the liquid under pressure for spraying.[6]

The original design of the ENS was intended for drift spraying locusts using dieldrin.[7] Of the spray volume, 90% was estimated to be within the band of 50–130 $\mu$m.[7] Other droplet size studies have been carried out[8,9] but we do not know of any published laser-sizing analyses of a standard ENS.

The Food and Agriculture Organisation (FAO) recommends that droplets should be produced between 40 and 120 $\mu$m.[10] Small droplets, less than 40 $\mu$m in diameter, are subject to exo-drift out of the target area. Droplets which are too large make inefficient use of the volumes of formulation applied at the ultra-low-volume rates of application.

Temperatures of up to 120°C are expected to be generated by the exhaust gases in the exhaust stack of the ENS.[11] Whilst the insecticide formulation is enclosed in a brass feed pipe within the centre of the exhaust stack it is possible that, after extended use, heat will be absorbed by the feed pipe and the insecticide will be exposed to similar temperatures of up to 120°C.

The viability of blastospores of *Verticillium lecanii* (A.W. Zimmerm.) Viegas was significantly reduced when applied using the 'Pulsfog K2' thermal fogger.[12] The exhaust gases of this equipment are water-cooled from 500°C to around 80°C but transient exposure to this temperature stress was sufficient to prevent the use of this thermal fogger for the application of wettable powder formulations of *V. lecanii*.[12] It has been established that *M. flavoviride* conidia are tolerant to moderate temperatures (60–80°C) provided moisture is removed from the formulation.[13] In the ENS, conidia of *M. flavoviride* will be exposed to temperatures of up to 120°C for 3–4 s. A decrease in the viability of the fungus resulting from such a temperature stress may reduce the efficacy of the formulation and limit the use of the sprayer as an application method for this mycopesticide.

In this paper we report on an evaluation of the Francome Fabrications MkII exhaust nozzle sprayer, as a piece of spray application equipment for oil-based formulations of *M. flavoviride*. The evaluation of the ENS will be presented both in terms of the droplet spectra produced and also in terms of the survival of the conidia passing through the sprayer.

## 2 METHODS

### 2.1 Investigation of the viability and virulence of conidia of *Metarhizium flavoviride* after exposure to the temperature stresses of the ENS

#### *2.1.1 Formulation of conidia*

The isolate of *M. flavoviride* used in this and all subsequent experiments was the International Mycological Institute (IMI) number 330189 obtained from *Ornithacris cavroisi* (Finot) (Orthoptera: Acrididae) in Niamey, Niger in 1988 and has been adopted as a standard isolate in most LUBILOSA experiments. To assess the effect of the sprayer on the viability and virulence of *M. flavoviride* a 100-fold dilution ($2{\cdot}5 \times 10^{10}$ conidia litre$^{-1}$) of field-rate suspension of live conidia was made up in a paraffinic oil, 'Shellsol' T (Shell Chemicals Ltd), and then filtered through a 150-$\mu$m sieve.

#### *2.1.2 Collection of conidia from the spray plume of the ENS*

This experiment was carried out in conjunction with the droplet analysis investigation. The ENS was attached to a 2·0 litre series III Petrol Landrover by a heavy-duty rubber exhaust pipe. For the safety of the operator, the vehicle was stationed in a doorway to allow free air movement and to prevent the build-up of potential toxic gases from the exhaust and engine. To prevent contamination of the environment with virulent conidia, the sprayed formulation was collected by a suction system positioned approximately 30 cm above the nozzle of the sprayer.

Three distinct nozzle types are available for use with the ENS. The number 1 nozzle has an internal diameter of 2·5 mm, the number 2 of 4·0 mm and the number 3 of 7·5 mm. During the collection of conidia from spray samples, the sprayer was fitted with the number 1 nozzle, the number 1 restrictor ring (internal diameter 12·5 mm) and operated as directed in the Francome Fabrications MkII operations manual (available from Francome Fabrications Ltd, Lowesden Works, Lambourn Woodlands, Berkshire, UK). This is the recommended set-up for locust control operations. Larger-diameter nozzle orifices increase the output and are known to produce larger droplets.[14] The number 1 nozzle, having the lowest output, therefore presents a 'worst case' by maximising the duration of the high temperature stress that the conidial formulation will experience within the exhaust stack of the sprayer.

At periods during this droplet spectra assessment, three short emissions of live conidial formulation were sprayed. The bursts of the conidial formulation were

timed to reflect the heating-up of the engine and of the sprayer. A sample of the conidial formulation was collected from the suction system during each burst, to serve as a treatment receiving an unquantified temperature stress during the operation of the sprayer. The bursts were made after 15, 60 and 120 min. A sample of unsprayed conidia from the tank of the ENS was taken as a control. Non-indicating silica gel was added to the samples, which were then placed in a refrigerator at 5(±1)°C for at least 24 h prior to testing. For each sample taken, external temperatures of different parts of the sprayer were recorded using an electronic temperature probe (Philips Instruments).

*2.1.3 Assessment of viability*

Samples of the sprayed and unsprayed conidia were plated out onto Sabouraud Dextrose Agar (SDA; Unipath Ltd, Basingstoke) and placed in an incubator at 25(±1)°C. After 24 and 48 h the SDA plates were removed from the incubator and examined under a compound microscope at a magnification of ×300 to score for germinating conidia. Where possible, at least 300 conidia were examined to record numbers of germinating and non-germinating individuals. A conidium was said to have germinated if the germ tube visibly projected to a length at least equivalent to the width of the conidia.[15]

*2.1.4 Assessment of virulence*

A bioassay was carried out on immature adult locusts of *S. gregaria* grown in culture at the International Institute of Biological Control (IIBC). Seven treatments were set up and applied to 20 locusts per treatment. The treatments are given in Table 1. Each treatment containing conidia was adjusted to a concentration of $4{\cdot}0 \times 10^9$ conidia litre$^{-1}$. A 2 μl inoculum of each treatment (except C), was introduced under the pronotum of each insect.[16] Each of the 140 locusts was placed in a separate plastic box lined with tissue paper and these were then incubated in a controlled environment chamber (33°C and approximately 35% RH) with constant light.

Each day during the incubation period of 14 days the locusts were examined for the presence of disease and death. The date and cause of death was recorded for each insect. The cause of death was recorded as either death due to fungus (*M. flavoviride*) or death due to a bacterial infection.

*2.1.5 Statistical analysis*

Statistical analysis was performed using GLIM 3.77 (Copyright, 1985 Royal Statistical Society, London).[17] In the assessment of viability, the data collected were proportion data; that is to say, the number of conidia germinating was expressed as a proportion of the total number of conidia examined. A model was fitted using binomial errors with a logit link function to ensure linearity. The number germinating was declared as the response variable with the total number of conidia examined declared as the binomial denominator to indicate the sample sizes. The maximal model was generated by the addition of the factor 'sample of conidia from the ENS' to the null model originally fitted to the data. Significance differences between the means of each of the factor levels were assessed by the method of contrasts.[17]

The analysis of the assessment of virulence was carried using a different technique.[17] Individuals still alive after 14 days were censored and a vector was set up to reflect this. This vector, the censoring indicator, was declared as the response variable with the error structure declared as Poisson. The link function was declared as the log link and the log of the time of death was declared as an offset to introduce the variation to be explained by the model. A model was fitted to the data and the factor 'treatment' was added to give the maximal model. Again, significant difference between

**TABLE 1**
Experimental Treatments Applied to the Locusts in a Laboratory Bioassay

| *Code* | *Treatment* |
|---|---|
| C | Adult locusts with no form of treatment other than handling to mimic the handling received by other locusts in the remaining treatments |
| 1 | Adult locusts inoculated with 2 μl of Shellsol T only |
| 2 | Adult locusts inoculated with 2 μl of Shellsol T with freshly harvested conidia of *M. flavoviride* |
| 3 | Adult locusts inoculated with 2 μl of the unsprayed sample of conidial formulation collected from the ENS |
| 4 | Adult locusts inoculated with 2 μl of the sample of conidial formulation collected after 15 min sprayer operation |
| 5 | Adult locusts inoculated with 2 μl of the sample of conidial formulation collected after 60 min sprayer operation |
| 6 | Adult locusts inoculated with 2 μl of the sample of conidial formulation collected after 120 min sprayer operation |

the means of each of the factor levels was assessed by the method of contrasts.

### 2.2 Analysis of the droplet spectra produced by the ENS

*2.2.1 Operational set-up*

The ENS was set up as in Section 2.1.2 and situated under a scaffolding designed to support the Malvern Series 2600cc particle size analyser (PSA). With the vehicle in neutral gear, the accelerator pedal was depressed to achieve a back pressure in the sprayer of 0·2 bar. This 0·2 bar was kept as a standard spray pressure for the entire droplet analysis. The nozzle tip was approximately 20 cm below the path of the laser and the centre of the plume passing through the laser was approximately 30 cm from the 300 mm detector lens.

*2.2.2 Effect of different formulations upon droplet spectra*

Blank formulations of 'Shellsol' T, and a more viscous naphthenic oil, 'Ondina' EL, (Shell Chemicals Ltd) were sprayed through each of the three nozzles described in Section 2.1.2. A blank mixture of 'Shellsol' T and 'Ondina' EL (50 + 50 by volume) was sprayed through both nozzles 1 and 2. Finally a field rate formulation ($2{\cdot}5 \times 10^{12}$ conidia litre $^{-1}$) of *M. flavoviride* suspended in a 50 : 50 mixture of 'Shellsol' T and 'Ondina' EL was sprayed through the number 1 and 2 nozzles only. This last formulation was made using killed conidia of *M. flavoviride* and is referred to in this paper as Green muscle.

The Malvern Series 2600cc PSA used in this experiment operates based on the principle of laser diffraction,[18] allowing droplet spectra for the combinations of nozzles and formulations detailed above to be easily described in terms of their volume median diameter (VMD). A measure of the usefulness of each droplet spectrum is also presented by a value of the mean percentage of droplets whose diameter is between 50 and 100 $\mu$m.[19] The greater the percentage the more useful the spray.

*2.2.3 Effect of nozzle height setting upon droplet spectra*

Under the same conditions as given in Section 2.2.1, a formulation of Green muscle was used to investigate the effect of the height setting of the number one nozzle with respect to the level of the number one restrictor ring in effecting variation in droplet spectra. Readings were taken using the Malvern Series 2600cc PSA corresponding to nozzle settings both above and below the level of the restrictor ring.

## 3 RESULTS

### 3.1 Investigation of the viability and virulence of conidia of *Metarhizium flavoviride* after exposure to the temperature stresses of the ENS

*3.1.1 External temperature readings*

The external temperatures of various parts of the ENS taken during the collection of the spray samples for viability assessments are presented in Table 2. As the engine was left to run, the external temperature of the sprayer increased to a maximum of 105·8°C at the base of the exhaust stack. There was no indication that temperatures in excess of the 120°C, previously recorded by Watts *et al.*[11] had been generated.

*3.1.2 Assessment of viability*

The viability of the conidia collected from the spray samples is presented in Fig. 1. The residual deviance of the maximal model for the germination tests indicated that the data were overdispersed. A correction was applied and significance testing was carried out by $F$-tests in place of the usual $\chi^2$-test.[17] After 24 and 48 h incubation there was no significant difference in viability between the M and L samples ($F_{1,16} = 2{\cdot}66$; $P > 0{\cdot}05$ and $F_{1,16} = 0{\cdot}78$; $P > 0{\cdot}05$ respectively). The germination success of sample E was significantly lower than that of M and L both after 24 and 48 h ($F_{2,16} = 18{\cdot}34$; $P < 0{\cdot}05$ and $F_{2,16} = 21{\cdot}56$; $P < 0{\cdot}05$ respectively). The unsprayed sample of conidia (U) had the greatest germination success after 24 and 48 h incubation ($F_{2,16} = 54{\cdot}4$; $P < 0{\cdot}05$ and $F_{2,16} = 27{\cdot}41$; $P < 0{\cdot}05$ respectively) indicating the sprayer had had a

**TABLE 2**
External Temperatures of Areas of the Spray Equipment During the Extended Running of the Exhaust Nozzle Sprayer

| | *Temperature (°C)* | | |
|---|---|---|---|
| *Running time (min)* | *Exhaust pipe (Landrover)* | *Exhaust chamber inlet (ENS)* | *Top of exhaust stack (ENS)* |
| 0 | 21·0 | 21·0 | 21·2 |
| 15 | 84·3 | 98·2 | 70·5 |
| 60 | 98·6 | 102·3 | 78·9 |
| 120 | 104·3 | 105·8 | 81·6 |

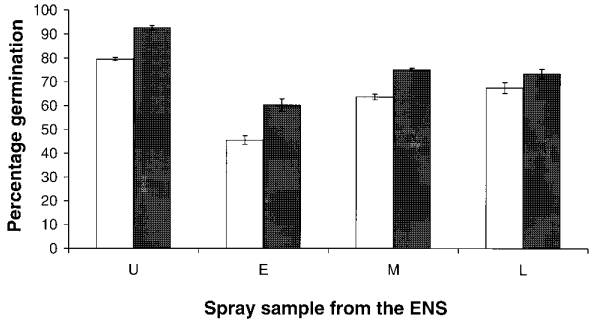


**Fig. 1.** Response of conidia of *M. flavoviride* to the stress of application by the exhaust nozzle sprayer. U: unsprayed conidia. E: spray sample taken after 15 min of sprayer operation. M: spray sample taken after 60 min of sprayer operation. L: spray sample taken after 120 min of sprayer operation. (□) 24 h (■) 48 h. Bar = 1 s.e.

significant detrimental effect on the viability of the conidia. There was no evidence of delayed germination resulting from exposure to the stresses of the ENS ($F_{3,18} = 0{\cdot}84$; $P > 0{\cdot}05$).

*3.1.3 Assessment of virulence*

The survivorship curves for locusts exposed to each treatment are presented in Fig. 2 and the average survival times are given in Table 3. The conidia from the spray samples (treatments 3, 4, 5 and 6) proved to be highly virulent against the immature adults of *S. gregaria*. These samples brought about the death of the locusts in 6–7 days. A few locusts in the spray treatments died from bacterial infections, but the majority died from *Metarhizium* infection. Control mortality (treatments C, 1 and 2) rose to about 50% by the end of the experimental period. Locust mortality in the control treatments was due to bacterial infection. None of the locusts in these three treatments died from *Metarhizium* infection. The conidia used to make up treatment 2 were subsequently found to be dead, explaining the result given in Fig. 2 where none of the locusts exposed to that treatment died from *Metarhizium* infection.

The average survival times of treatments 3–6 were not significantly different from each other and the average survival times of treatments C, 1 and 2 were not significantly different from each other ($\chi^2 = 0{\cdot}741$, 5 degrees of freedom; $P > 0{\cdot}05$). However, there was a highly significant difference between the average survival times of the two groups ($\chi^2 = 36{\cdot}96$, 1 degree of freedom; $P < 0{\cdot}001$).

**TABLE 3**
Average Survival Times for Locusts Challenged with a Range of Treatments in a Laboratory Bioassay as detailed in Table 1

| *Treatment* | *Average survival time (days) (±1 s.e.)* |
|---|---|
| C | 9·5 (±0·91) |
| 1 | 9·91 (±0·81) |
| 2 | 12·18 (±0·82) |
| 3 | 6·85 (±0·11) |
| 4 | 6·45 (±0·27) |
| 5 | 6·3 (±0·11) |
| 6 | 7·95 (±0·37) |

## 3.2 Analysis of the droplet spectra produced by the ENS

*3.2.1 Effect of different formulations upon droplet spectra*

The results of the investigation into the droplet spectra produced by the three nozzles of the ENS in response to different oil formulations are presented in Table 4. Regression analysis for the entire data set, pooling all nozzles together, indicates that there does not appear to be an effect of viscosity ($r^2 = 0{\cdot}067$: $F_{1,26}$-1·79; $P > 0{\cdot}05$). Similarly for nozzle number 1 alone there is no effect of viscosity upon the VMD ($r^2 = 0{\cdot}041$: $F_{1,13} = 0.56$; $P > 0{\cdot}05$). Whilst the VMDs of the droplet spectra for formulations of pure 'Ondina' EL were in general larger than those from formulations of 'Shellsol' T, for all nozzles, the 50 : 50 mixture of the two oils, with an intermediate viscosity, produced on average larger droplets than both formulations under the same conditions.

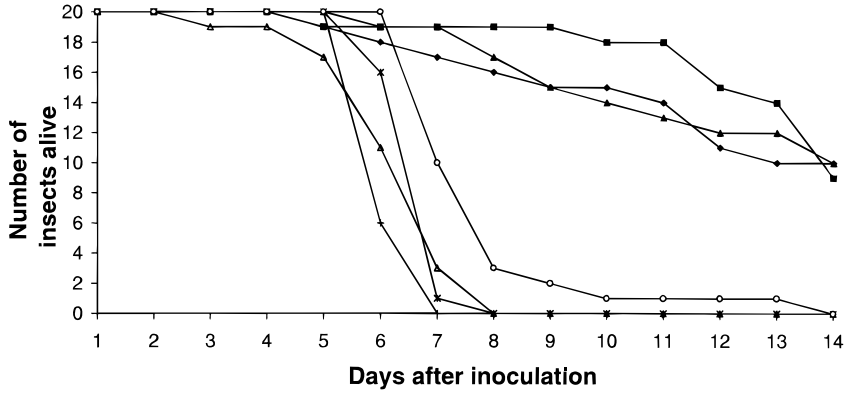


**Fig. 2.** Survivorship curves for locusts in a bioassay challenged by conidia of *Metarhizium flavoviride* from formulations sprayed through the exhaust nozzle sprayer. (◆) Control, (▲) 1, (■) 2, ( + ) 3, (∗) 4, (△) 5, (○) 6.

**TABLE 4**
Droplet Spectra for Three Nozzles of the Exhaust Nozzle Sprayer for a Range of Formulations as Calculated by Laser Analysis

| *Nozzle type* | *Formulation* | *Viscosity (mPa at 20°C)* | *Mean VMD (±s.e.)* | *Mean % in 50–100 μm band (±s.e.)* | *Number of replicates* |
|---|---|---|---|---|---|
| 1 | Shellsol T | 1·8 | 27·4 (±1·2) | 15·7 (±1·7) | 7 |
| 1 | Ondina EL | 31 | 40·1 (±5·6) | 27·4 (±2·2) | 4 |
| 1 | 50 : 50 mixture | 6 | 61·0 (±11·9) | 31·5 (±1·9) | 2 |
| 1 | Green muscle | 7–8[a] | 55·0 (±0·8) | 32·6 (±0·6) | 2 |
| 2 | Shellsol T | 1·8 | 41·7 (±0·02) | 27·4 (±0·3) | 2 |
| 2 | Ondina EL | 31 | 40·2 (±0·3) | 28·8 (±0·7) | 2 |
| 2 | 50 : 50 mixture | 6 | 47·7 (±2·4) | 29·7 (±0·9) | 2 |
| 2 | Green muscle | 7–8[a] | 51·6 (±1·9) | 30·5 (±0·2) | 2 |
| 3 | Shellsol T | 1·8 | 78·0 (±10·2) | 26·8 (±1·8) | 2 |
| 3 | Ondina EL | 31 | 99·8 (±2·3) | 26·3 (±0·01) | 2 |

[a] Green muscle is a particulate suspension therefore the viscosity is given as a range to reflect variation depending upon the method of measurement.

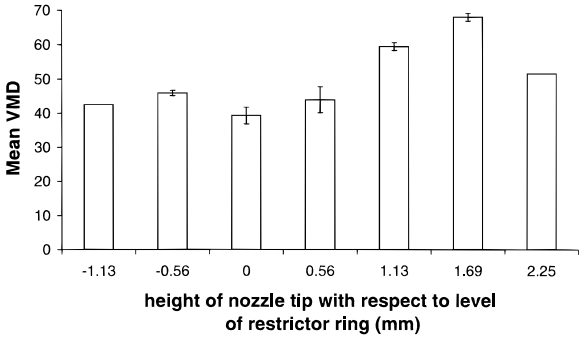


**Fig. 3.** Droplet spectra produced under different height settings of the number 1 nozzle at a spray pressure of 0·2 bar using dead conidia formulated as field rate Green muscle. Negative height settings refer to positions of the nozzle tip below the level of the restrictor ring and positive settings refer to those above the level of the restrictor ring. Bar = 1 s.e.

Each record was taken using the same nozzle setting and therefore variation in the results may reflect differences in spray pressures generated by the Landrover engine. Some of the variability may come from contamination between formulations in the fluid feed pipe, but this could not be tested.

*3.2.2 Effect of nozzle height setting upon droplet spectra*
The effect of the different height settings for nozzle 1 upon VMD values is given in Fig. 3. The position of the nozzle tip with respect to the level of the restrictor ring is an important factor in determining the VMD for the droplet spectra of that nozzle. Positioning the nozzle tip below or at the level of the restrictor ring produces little effect on the resulting VMD. Only as the nozzle projects out above the level of the restrictor ring does the VMD

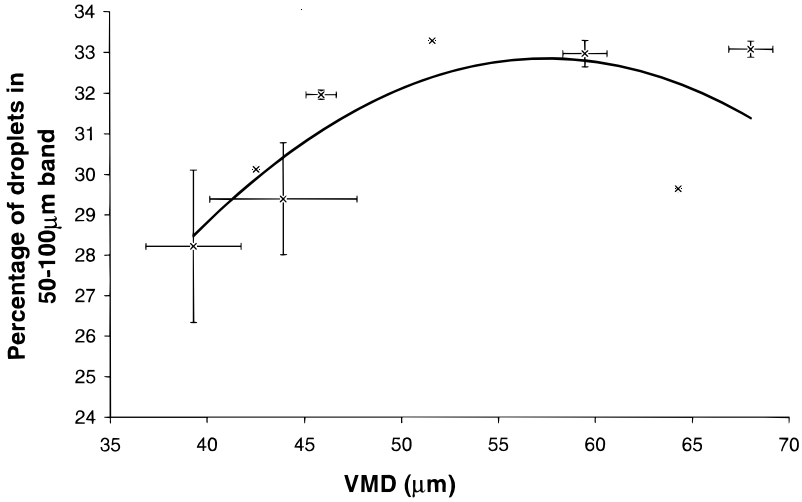


**Fig. 4.** Percentage of spray droplets in the 50–100 μm band given as a function of the VMDs obtained during the nozzle height setting experiment ($y = -0{\cdot}01x^2 + 1{\cdot}52x - 10{\cdot}86$; $R^2 = 0{\cdot}54$). Bars = 1 s.e.

increase. If the nozzle projects too far, the VMD begins to decrease again.

In Fig. 4, plots are presented of 'mean percentage of droplets in the 50–100 mm band' against VMD and a trend line has been inserted to illustrate the relationship between these two factors. The function described in Fig. 4 revealed that, for a field-rate formulation of Green muscle delivered *via* the number 1 nozzle at 0.2 bar, a maximum of 33% of droplets in the 50–100 $\mu$m band was achieved for a VMD of approximately 58 $\mu$m. From Fig. 3, a spray VMD of 58 $\mu$m will be created by nozzle 1 when the nozzle is projecting above the level of the restrictor ring by between 1 and 2 mm, when similar formulations are sprayed under a back pressure of 0·2 bar. Beyond the limits presented here the quality of the spray deteriorates and a much greater proportion of droplets that are either too large or too small is produced.

## 4 DISCUSSION

A reduction in germination success of approximately 30% was recorded for conidia sprayed through the ENS. Therefore the sprayer is having a detrimental affect on the viability of conidia in the 'Shellsol' T formulation. The reason for this is unclear. Possibly the temperatures in the exhaust stack of the ENS were responsible for the loss in viability. However, in the laboratory, conidia of *M. flavoviride* from the same harvest as those used in this experiments with the ENS could tolerate up to 30 s exposure to 150°C and maintain over 80% germination success.[14] The key factor in maximising temperature tolerance of *M. flavoviride* is to ensure that the moisture content of the conidia is kept as low as possible. Adding non-indicating silica gel to dry conidia in storage and to the oil-based formulations prior to spraying results in an unquantified drying, but is essential for formulation stability.[15] In such a dry state the temperature tolerance of conidia is sufficient to withstand any temperature stress that might be experienced in the ENS.

In addition to the temperature of the exhaust gases, their chemical composition may have in some way affected the conidia. In the tank where the exhaust gases provide the back pressure to propel the formulation, the viability of the conidia appears unaffected and the effect must therefore happen outside of the spray tank at the nozzle tip. The nozzle tip will be the site of a number of stresses all of which could contribute, either individually or in interactions, to the observed reduction in conidial viability. The physical and chemical nature of the exhaust gases will be important factors affecting conidial viability but the potential for photodegradation of the conidia upon exposure to UV light should not be overlooked.

All of the samples of spray from the ENS killed locusts in the bioassay. Although germination success had been reduced after passing through the ENS, this did not correlate with a reduction in virulence of the conidia against the insect host. It has been demonstrated how an order of magnitude difference in the concentration of a conidial formulation would only result in less than one day's difference in the average survival times for treated locusts in bioassays.[20] The fall in viability observed in the germination test is equivalent to reducing the effective concentration of conidia in the formulations (from $4{\cdot}0 \times 10^9$ conidia litre$^{-1}$ to $2{\cdot}8 \times 10^9$ conidia litre$^{-1}$). The similarity of the average survival times for each of the spray treatments would therefore only have been expected if virulence had been unaffected during the operation of the ENS.

Freshly harvested conidia formulated in peanut oil and administered in a 2-$\mu$l dose of concentration $3{\cdot}8 \times 10^{10}$ conidia litre$^{-1}$ would achieve an average survival time of 5($\pm$0·08) days for *S. gregaria* in a locust bioassay.[21] Whilst the spray samples acted more slowly than this, the speed of action is fast enough to suggest that exposure to the ENS in any form does not reduce the virulence of conidia to any appreciable degree. The difference between the data from the peanut oil experiment and those presented here reflects differences in the bioassays performed. For example different cohorts of locusts were used, different oil formulations were used. However, probably by far the most important difference is the concentration of spores administered to the locusts. The locusts in this bioassay were challenged by a formulation with a concentration of $4 \times 10^9$ conidia litre$^{-1}$ as compared to $3{\cdot}8 \times 10^{10}$ conidia litre$^{-1}$ administered in the peanut oil experiment. The more dilute the conidial concentration the longer the average survival time of the target insects is expected to be.

The setting of the number 1 nozzle tip with respect to the level of the restrictor ring is important but not crucial. Provided the nozzle tip is between 1 and 2 mm above the level of the restrictor ring the optimum droplet spectrum will be produced. The recommended height setting by the manufacturer lies below the settings recommended here. The manufacturers recommend a nozzle settings of 0·5–1 mm above the level of the restrictor ring. Using a similar model of Landrover and a spray pressure of 0·28 bar these conditions are said to produce an VMD of 69 $\mu$m; however, the manufacturer gives no indication of the variation of these droplet spectra. Without data on the source of the manufacturer's recommendations, no direct comparison with the nozzle settings in this paper can be made.

As the height setting of the number 1 nozzle was altered a range of VMDs between 38 and 70 $\mu$m was produced. Most of these VMDs fall within the preferred range of 50–100 $\mu$m, but the actual proportion of droplets in each spray within this band was very low. The maximum percentage of droplets in the required band

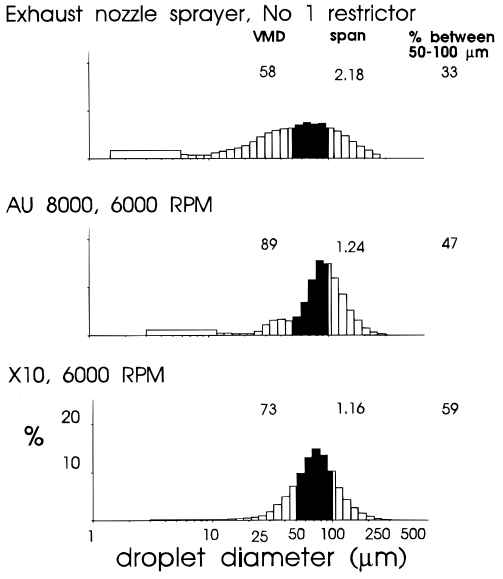


**Fig. 5.** A comparison of the droplet spectra for field rate formulations of Green muscle achieved by the exhaust nozzle sprayer, the Micronair AU8000 and the Micron X10.

was 33% and this corresponded to a VMD of approximately 58 μm. The greater proportion of each spray was made up of droplets lying outside this range and, as such, of a poor quality for spraying locusts.

In Fig. 5 the droplet spectrum of the ENS is compared to the published spectra produced by two other ultra-low-volume vehicle-mounted sprayers commonly used in locust control operations.[22] These two sprayers (the Micronair AU8000 spinning cage atomiser and the Micron X10 spinning disc atomiser) use controlled droplet application (CDA) technology, that is to say they are designed to produce droplets with a specific and controllable size. With this technology a greater percentage of the droplets can be delivered within the required size band of 50–100 μm. Consequently, less formulation is wasted in droplets that are too small or too large, and better targeting of the locust is achieved. The best CDA equipment should produce 80% or more of the droplets within the 50–100 μm size band.[19]

The quality of droplets produced by the ENS is inferior to the spectra produced by rotary atomisers and the machines can be difficult to calibrate. However they have a reputation for robustness and a number of countries which suffer serious locust problems still rely heavily on the use of the ENS in their control programmes.[23,24] Unless these countries adopt CDA technology the ENS will remain the principal means of applying pesticides to locusts. These and other countries have expressed interest in the potential environmental benefits of myco-insecticides.

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